Serum Anion Gap: Its Uses and Limitations in Clinical Medicine

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The serum anion gap, calculated from the electrolytes measured in the chemical laboratory, is defined as the sum of serum chloride and bicarbonate concentrations subtracted from the serum sodium concentration. This entity is used in the detection and analysis of acid-base disorders, assessment of quality control in the chemical laboratory, and detection of such disorders as multiple myeloma, bromide intoxication, and lithium intoxication. The normal value can vary widely, reflecting both differences in the methods that are used to measure its constituents and substantial interindividual variability. Low values most commonly indicate laboratory error or hypoalbuminemia but can denote the presence of a paraproteinemia or intoxication with lithium, bromide, or iodide. Elevated values most commonly indicate metabolic acidosis but can reflect laboratory error, metabolic alkalosis, hyperphosphatemia, or paraproteinemia. Metabolic acidosis can be divided into high anion and normal anion gap varieties, which can be present alone or concurrently. A presumed 1:1 stoichiometry between change in the serum anion gap (ΔAG) and change in the serum bicarbonate concentration (ΔHCO3−) has been used to uncover the concurrence of mixed metabolic acid-base disorders in patients with high anion gap acidosis. However, recent studies indicate variability in the ΔAG/ΔHCO3− in this disorder. This observation underscores the ability to use this ratio alone to detect complex acid-base disorders, thus emphasizing the need to consider additional information to obtain the appropriate diagnosis. Despite these caveats, calculation of the serum anion gap remains an inexpensive and effective tool that aids detection of various acid-base disorders, hematologic malignancies, and intoxications.


Determinants of the Serum Anion Gap

Although the anion gap can be calculated using serum or plasma electrolytes, most frequently serum values are used. Therefore, our discussion is restricted to the serum anion gap, with the understanding that the anion gap that is calculated using either serum or plasma values is acceptable.

Gamble (9), in his short lecture syllabus Chemical Anatomy, Physiology, and Pathology of the Extracellular Fluid, was one of the first individuals to emphasize the importance of charge balance in the evaluation of perturbations in the ionic environment of the blood and other body fluids. A graphic display of the ionic environment of the serum, often termed a “gamblegram,” is depicted in Figure 1 and illustrates the concept that total serum cations and total serum anions must always remain equal to ensure charge balance. Therefore, the sum of circulating cations—sodium, potassium, calcium, magnesium, and cationic proteins—must equal the sum of circulating anions—chloride, bicarbonate, anionic proteins, inorganic phosphate, sulfate, and organic anions:

\[
\begin{align*}
Na^+ + K^+ + Ca^{2+} + Mg^{2+} + \text{Protein}^+ &= Cl^- + HCO_3^- \\
+ \text{Protein}^- + HPO_4^{2-} / HP0_4^- + SO_4^{2-} + OA^- 
\end{align*}
\]

However, routinely, only the cations sodium and potassium and the anions chloride and bicarbonate are measured; there-
constituents of the anion gap (13,14). Specifically, sodium and potassium have altered their methods of measuring the individual constituents measured in mEq/L varied from 11 to 16 (range 10 to 20) (10) to 12 ± 4 (range 8 to 16) (1-3,12). These values were based on sodium concentration determined by flame photometry, chloride concentration by a colorimetric method (usually mercuric-nitrate thiocyanate), and total CO₂ content by acidification of the specimen followed by colorimetric titration. Subsequently, many clinical laboratories have altered their methods of measuring the individual constituents of the anion gap (13,14). Specifically, sodium and chloride often are measured by ion selective electrodes. Total CO₂ content is determined either by the rate of pH change determined with a pH electrode or, more frequently, by an enzymatic method, which measures the conversion of NAD to NADH. With these methods, values for sodium deviate only slightly from normal values on the basis of standards used, whereas chloride measurements can be substantially greater than previous normal values (13). As a consequence, the mean serum anion gap in many clinical laboratories that use ion selective electrodes is lower than reported previously, averaging 6 ± 3 mEq/L (13,15). Indeed, in one study, two separate laboratories that used these methods reported that 62 and 79% of healthy individuals had a serum anion gap of 6 mEq/L or less (13). Some laboratories have lowered the calibration set point for chloride so that the value for serum chloride and, therefore, the serum anion gap is similar to that initially described in the 1970s (13). Thus, the average anion gap and range of normal values will vary in different health care facilities, and it is essential that the clinician know the range of normal for the particular clinical laboratory to assess properly the changes in the serum anion gap. Furthermore, whichever methods are used for measurement of electrolytes, there is a wide range of normal values for the serum anion gap (reported normal values vary by as much as 6 to 10 mEq/L in individual clinical laboratories) (1,10,13,16), reflecting great interindividual variability. This variability, of course, must reflect the relatively wide range of normal values of each of its constituents. Moreover, because the negative charges of albumin make up the bulk of the unmeasured anions, variability in the serum concentration of albumin within the normal range (3.8 to 5.1 g/dl) also will contribute to the biologic variability of the anion gap (16,17). Given this wide interindividual variability, it is important, if possible, to know the prevailing baseline value of the serum anion gap for a particular individual. If the baseline serum anion gap of an individual is not known and the range of normal values of a particular laboratory is used to assess the anion gap, then it is possible that disorders that cause deviations in the serum anion gap might not be recognized because they are insufficient to shift the serum anion gap outside the normal range.

Deviations in the Serum Anion Gap

Deviations from the prevailing normal value of the serum anion gap can reflect either errors in the measurements of its constituents or changes in the concentrations of unmeasured cations and/or anions.

Low or Negative Serum Anion Gap

A serum anion gap that is below the lower limits of normal is a relatively infrequent occurrence. Its prevalence ranged from 0.8 to 3% of more than 80,000 total sets of electrolytes that were obtained in two large clinical laboratories (18-20). The relatively infrequent occurrence of a low anion gap might reflect, in part, the wide range of the normal serum anion gap. As noted previously, factors that cause a reduction in its value might not be sufficient to cause it to fall outside the normal range. Indeed, in one study, approximately 80% of the low
values were <4 mEq/L below the lower limits of normal (20). A negative serum anion gap is even more uncommon: 0.12% of all sets of electrolytes that were obtained during a 12-mo period in a large clinical laboratory (20). Disorders that are associated with a low or negative serum anion gap are listed in Table 1.

**Laboratory Error.** Accurate calculation of the serum anion gap requires accurate measurement of the electrolytes that are necessary for its determination, including sodium, chloride, and bicarbonate. In more than 67,000 calculations of the serum anion gap that were based on consecutive sets of electrolytes measured at the Massachusetts General Hospital (18), a low serum anion gap was observed in <1%. Of these low values, >90% were attributed to a laboratory error in measurement of one of the electrolytes.

In this regard, there is controversy about the use of the anion gap as a means of quality control. Buckley-Sharp and Miller (10) found that it was a poor substitute for quality control of its individual constituents. By contrast, Witte et al. (1) suggested that the serum anion gap provided a tool of comparing results of multiple determinations on a given patient, thereby offering an additional means of quality control. At this time, when a low value for serum anion gap is obtained, many clinical laboratories will repeat measurement of its constituents. However, most clinical laboratories do not depend on the serum anion gap to detect systematic or random errors in chemical determinations; rather, they depend primarily on determinations of individual electrolytes using reference samples (1,10,13).

Although random errors can occur, specific abnormalities can predispose to consistent errors in the calculation of the anion gap. An underestimation of serum sodium concentration as measured by flame photometry can occur in the presence of hypernatremia, thereby leading to spuriously low or negative values for the serum anion gap (15). This error is avoided by use of ion selective electrodes. If an indirect method that requires predilution is used to measure sodium, then errors in calculation of the serum anion gap will arise with severe hypertriglyceridemia or dysproteinemia as a result of underestimation of serum sodium concentration that is caused by dilution artifacts even with ion selective electrodes (21,22). With severe macroglobulinemia, the measured sodium concentration can be as much as 23 mEq/L lower than the actual value (22). This effect seems to be related to incorrect aspiration or sampling.

Errors in measurement of serum chloride, which are more common when it is determined by the colorimetric method, also will cause an erroneous value for the serum anion gap (21). In the presence of marked hypertriglyceridemia, light-scattering effects can produce marked overestimation of serum chloride and therefore a spuriously low serum anion gap (21). If serum chloride is determined by direct ion selective electrodes, however, then the measured value will be very close to the actual value, despite hypertriglyceridemia. Similar to the effects of sodium, deviations from the correct value can occur with ion selective electrodes if indirect methods that require predilution are used.

Serum bicarbonate concentration is determined in many clinical laboratories by acidifying the specimen and measuring the total carbon dioxide released. If the serum is not separated from

<table>
<thead>
<tr>
<th>Table 1. Causes of a low or negative serum anion gap</th>
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<tbody>
<tr>
<td><strong>Low serum anion gap</strong></td>
</tr>
<tr>
<td>laboratory error</td>
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<tr>
<td>underestimation of serum sodium</td>
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<tr>
<td>overestimation of serum chloride</td>
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<tr>
<td>overestimation of serum bicarbonate</td>
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<tr>
<td>hypoalbuminemia</td>
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<tr>
<td>monoclonal IgG gammapathy</td>
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<tr>
<td>polyclonal gammopathy</td>
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<tr>
<td>bromide intoxication</td>
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<tr>
<td>lithium intoxication</td>
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<tr>
<td>hypercalcaemia</td>
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<tr>
<td>hypermagnesemia</td>
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<tr>
<td>polymyxin B</td>
</tr>
<tr>
<td>iodide intoxication</td>
</tr>
<tr>
<td><strong>Negative serum anion gap</strong></td>
</tr>
<tr>
<td>laboratory error</td>
</tr>
<tr>
<td>bromide intoxication</td>
</tr>
<tr>
<td>multiple myeloma</td>
</tr>
<tr>
<td>iodide intoxication</td>
</tr>
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</table>
cellular elements soon after collection, then leukocytes will continue to produce carbon dioxide in vitro, falsely elevating serum bicarbonate and producing a spurious reduction in serum anion gap by as much as 2 to 3 mEq/L (23).

**Hypoalbuminemia.** Under normal conditions, the bulk of the serum anion gap (approximately 80%) is due to the sum of the anionic charges on circulating proteins (16,17). Albumin is the most abundant of circulating proteins; therefore, changes in the concentration of serum albumin would be expected to alter the serum anion gap. Figge et al. (24) examined the impact of changes in serum albumin concentration on the serum anion gap of 152 patients who were admitted to an intensive care unit. Using computer analysis of the data, they determined that there was a direct correlation between changes in serum albumin concentration and the serum anion gap: For each 1-g/dl decrement in the serum concentration of albumin, the serum anion gap was decreased by 2.5 mEq/L. However, Carvounis and Feinfeld (25) found that each 1-g/dl decrement in serum albumin caused a reduction in the serum anion gap of 1.5 to 1.9 mEq/L. To evaluate this issue further, Feldman et al. (17) examined the relationship between serum albumin and serum anion gap in more than 5000 consecutive patients, more than 1100 of whom had a serum albumin concentration below the normal range and 420 of whom had a serum albumin concentration above the normal range. Similar to the results of Figge et al. (24), there was a direct correlation between serum albumin concentration and the serum anion gap: For every 1-g/dl decrease in serum albumin concentration, the serum anion gap fell by approximately 2.3 mEq/L. Also, it was increased by a similar amount with each 1-g/dl increment in serum albumin above the normal range. As a whole, these studies indicate a directional 2.3- to 2.5-mEq/L change in the serum anion gap for every 1-g/dl change in serum albumin. Because of the wide range of normal for the serum anion gap, even severe hypoalbuminemia (serum albumin concentration <1 g/dl) might not produce a serum anion gap below the normal range. However, hypoalbuminemia is the second most common cause of a low serum anion gap observed clinically (17,18,20).

**Monoclonal and Polyclonal Gammopathy.** The excess accumulation in blood of proteins that harbor a positive or negative charge at physiologic pH can cause alterations in the serum anion gap (4). Therefore, overproduction of monoclonal immunoglobulins (paraproteinemia), such as IgG and IgA, will cause deviations in the serum anion gap (4,26). IgG tends to be cationic, whereas IgA tends to be anionic (4,5,26). As a consequence, patients with IgG myeloma will tend to have a lower serum anion gap than normal (4,26). The serum anion gap in these patients can be slightly below the mean value for the laboratory, below the normal range, or rarely even be negative. There is a strong inverse correlation between the level of the serum anion gap and the concentration and net positive charge of IgG paraproteins. Therefore, negative values or values below the normal range are associated with the highest concentrations of paraproteins (5,26,27). Furthermore, reductions in the concentration of paraproteins after treatment are associated with normalization of the serum anion gap (28).

The serum anion gap also can be reduced in the presence of polyclonal gammopathy. Observations in 206 patients with polyclonal IgG gammopathy showed that the serum anion gap was lower by >50% compared with values that were obtained in 63 normal control subjects (6.4 ± 1.2 versus 15.4 ± 2.4 mEq/L) (29,30). Indeed, given the frequency of polyclonal gammopathy, this might be a more common cause of a low serum anion gap than myeloma. A negative serum anion gap, although theoretically possible, has not yet been reported in association with polyclonal gammopathy.

**Bromide Intoxication.** Bromide is a negatively charged halide, similar to chloride. It used to be present in nonprescription medications such as Bromo-Seltzer but now is present only in sedative drugs, pyridostigmine bromide that is used in the treatment of myasthenia gravis, and some herbal medications. Abnormally high concentrations of bromide in the blood can cause protein dermatologic, psychiatric, and neurologic symptoms (7). Although bromide is negatively charged and, therefore, its accumulation theoretically should increase the serum anion gap, it actually leads to a reduced or even negative serum anion gap (7,31). This effect occurs because bromide interferes with the measurement of chloride, causing a spurious elevation in its concentration. Serum chloride concentration was raised by approximately 3 mEq/L for every 1-mEq/L increase in the concentration of bromide when measured with the commonly used Kodak Ektachem 700 automated analyzer (an ion-selective electrode-based system; Rochester, NY) (31). Although the spurious elevation in chloride concentration is most pronounced with the use of an ion selective electrode, it also can be seen with all other methods that are used to measure chloride (7,32). The magnitude of reduction in the serum anion gap is related directly to the concentration of bromide in serum with values ranging from only slightly depressed to as low as ~60 mEq/L (33); however, a low serum anion gap can be observed with serum bromide concentrations well within the therapeutic range (7,34). After laboratory error, marked accumulation of bromide in the blood is the most frequent cause of a negative serum anion gap reported in the literature (20,21,33–35). However, given the less frequent presence of bromide in medications today, other causes of a negative anion gap are more likely. Despite this, in patients with a negative serum anion gap, bromide intoxication should be considered as a possibility and excluded if other causes are not obvious.

**Lithium.** Lithium is commonly prescribed for the treatment of bipolar disorder (6). Because lithium is a cation, it can lower the serum anion gap when present in sufficient concentration (6,36). Although a slightly reduced serum anion gap can be observed with a serum concentration of lithium as low as 0.5 to 1 mEq/L, values within the therapeutic range, a substantial decrease in the serum anion gap is observed more commonly when lithium concentration is >4 mEq/L (6). In individuals with clinical manifestations that are consistent with lithium toxicity, a decreased serum anion gap can be a clue to its presence.

**Hypercalcemia and Hypermagnesemia.** Marked increments in the level of cations that normally are present in serum but not used to calculate the serum anion gap, such as calcium and magnesium, theoretically can lower the serum anion gap.
Oster et al. (37) examined the effects on the serum anion gap of hypercalcemia that is associated with malignancy and that is caused by primary hyperparathyroidism. When values for both groups were considered together, there was no reduction in the serum anion gap compared with normal control subjects. However, in patients with hypercalcemia as a result of primary hyperparathyroidism, the serum anion gap was reduced by approximately 2.4 mEq/L, although the mean serum calcium concentration was actually lower than that in patients with malignancy-associated hypercalcemia (12.1 ± 0.1 versus 13.3 ± 0.3 mg/dl, respectively). Review by these investigators of several previously published studies that encompassed approximately 180 patients also revealed that the serum anion gap tended to be lower in patients with primary hyperparathyroidism, although the explanation for this difference was not evident. However, these findings suggest that the occurrence of a low serum anion gap in a patient with hypercalcemia could point to the presence of hyperparathyroidism.

The same investigators examined the effect on the serum anion gap of an increase in serum magnesium that is produced by administration of magnesium-containing compounds (36). Despite modest hypermagnesemia (4.1 ± 0.2 mEq/L), the mean serum anion gap was not altered from baseline (11.7 ± 0.7 versus 10.8 ± 0.5 mEq/L). Moreover, of the 15 patients with paired values for the serum anion gap, it increased in five, it decreased in one, and it remained unchanged in the others. These findings were explained, in part, by retention in the blood of sulfate, an unmeasured anion that is given along with magnesium: The retained unmeasured cations and anions canceled out each other. This group also reported a patient who had a serum magnesium concentration >9 mEq/L as a result of ingestion of large quantities of magnesium citrate and in whom no reduction in the serum anion gap was found (baseline anion gap: 11 mEq/L, Mg 2.2 mEq/L; anion gap: 14 mEq/L, Mg 9.6 mEq/L) (38). These observations indicate that hypermagnesemia should not be considered strongly in the differential diagnosis of a low or negative serum anion gap.

Miscellaneous Disorders. Dilution of the blood produces a proportional reduction in the serum sodium, chloride, and bicarbonate, causing only a slight fall in the serum anion gap, rarely ≥2 mEq/L (39). If sodium concentration is reduced but bicarbonate concentration remains unchanged, as has been reported for the syndrome of inappropriate antidiuretic hormone secretion (SIADH), then the serum anion gap could be lowered to a greater extent, even to zero (7,39). Indeed, Decaux et al. (39) described 16 patients who had hyponatremia that was associated with the SIADH and in whom the average serum anion gap was reduced by 27%, whereas serum sodium was decreased by only 16%. Patients with other disorders that are associated with hyponatremia of a similar magnitude had a far smaller change in the anion gap. Theoretically, then, a low serum anion gap in an individual with hyponatremia is consistent with the presence of the SIADH.

The antimicrobial polymyxin B, which is used for treatment of serious Gram-negative infections, possesses polycationic properties. It has been reported to produce a low or even negative serum anion gap (40). In vitro studies indicate that a low serum anion gap is observed when the formulation contains chloride rather than sulfate, because the retained negatively charged sulfate will counterbalance the positive charges of polymyxin, thereby preventing a change in the anion gap (40).

Iodide is a halide similar to chloride and bromide. Like bromide, it interferes with the measurement of serum chloride, giving an apparent halide concentration that is greater than the actual level of total halide in the blood. A low or negative serum anion gap has been described with abnormal concentrations of iodide (8). It remains uncertain, however, whether iodide and bromide act by an identical mechanism, because the spurious effect of iodide ingestion on the serum anion gap has been observed in the presence of very low serum concentrations of iodide (e.g., <1 mEq/L). Because iodide intoxication can be difficult to diagnose, a low or negative serum anion gap can be an important clue to its presence.

In summary, a low serum anion gap is a relatively uncommon occurrence, most frequently the result of laboratory error or severe hypoalbuminemia (17,18,20). Besides hypoalbuminemia, polyclonal gammopathy and monoclonal gammopathy with excessive accumulation of cationic IgG are the most common clinical disorders associated with a low serum anion gap (4,5,29,30). Therefore, once laboratory error and hypoalbuminemia have been excluded, a search for accumulation of IgG should be initiated. In patients with disturbed mentation or unexplained clinical findings, the possibility of lithium ingestion, bromism, or iodide intoxication should be considered. When the serum anion gap is negative in the absence of laboratory error, an extremely uncommon situation, bromide intoxication, iodide intoxication, and myeloma should be excluded (8,27,33).

High Serum Anion Gap

By contrast to a low serum anion gap, an elevated serum anion gap is a relatively common occurrence, particularly among hospitalized patients (19). Examination of more than 6000 sets of laboratory values from patients who were hospitalized at Ramathibodi Hospital during a 1-yr period revealed an elevated serum anion gap in approximately 37% (19). Also, 13% of 671 consecutive patients who were seen in a busy emergency department of an urban hospital had an elevated serum anion gap (41).

Disorders that are associated with an elevated anion gap are shown in Table 2. The most common disorder that is associated with an elevated serum anion gap is metabolic acidosis, specifically that due to the overproduction or decreased excretion of acid (2,3). More rare causes of an elevated serum anion gap include laboratory error (1), accumulation of anionic paraproteins (4), metabolic alkalosis (42), and severe hyperphosphatemia (43). As with a low serum anion gap, factors that tend to elevate the serum anion gap can be present but be insufficient to cause it to fall outside the normal range.

Metabolic Acidosis. The serum anion gap has found its greatest utility in the differential diagnosis of metabolic acidosis (2,3,44). Many of the physiologically important acids consist of one or more protons and an associated anion. If the acid that
accumulates in blood is hydrochloric acid, then no change in the serum anion gap would be expected, because an equivalent number of chloride ions are retained in the blood to maintain charge neutrality when bicarbonate ions are titrated by the retained protons. This type of metabolic acidosis, therefore, is termed normal anion gap or hyperchloremic metabolic acidosis. Actually, the serum anion gap in patients with hyperchloremic acidosis might not always remain constant but can fall by as much as 4 mEq/L if the acidemia is severe. This fall in the anion gap represents a change in the plasma-protein equivalency; the increased quantity of protons titrates plasma proteins, reducing their net anionic equivalency (45). A normal anion gap or hyperchloremic acidosis can result from several different mechanisms.

Substances such as cationic amino acid chloride salts and ammonium chloride are metabolized by the liver to hydrochloric acid. The loss of bicarbonate in the urine (occurring with various types of renal tubular acidosis or chronic renal failure) or the stool also can produce this electrolyte pattern. It has been postulated that the loss of bicarbonate, along with its counterbalancing cation sodium, produces volume contraction, thereby stimulating the renal tubule to retain sodium chloride. The consequences of these events are the replacement of sodium bicarbonate by sodium chloride. Loss of potential base in the form of organic acid anions also plays a role in the appearance of normal anion gap acidosis in the course of several overproduction acidoses. Because the mechanisms that produce normal anion gap or hyperchloremic acidosis have not been well delineated, it is possible that changes in the reabsorption of chloride independent of sodium or bicarbonate may contribute to the development of hyperchloremic acidosis. This possibility remains to be explored.

A reduction in distal tubular proton secretion, in the absence of or out of proportion to reductions in GFR, also can lead to normal anion gap acidosis. Although not proved, it has been speculated that normal anion gap acidosis results from the following sequence of events: Metabolism of ingested food produces sulfuric, phosphoric, and organic acids. Synthesis of new bicarbonate by the kidney, as measured by an increase in net acid excretion, neutralizes the acid, resulting in neutral acid balance. The impairment in renal collecting duct proton secretion and/or ammonia production in chronic renal failure reduces ammonium excretion and titratable acid excretion, causing net acid excretion to fall below acid production with resultant hypobicarbonatemia. A portion of the filtered sulfate and phosphate therefore are excreted with the cations sodium and potassium. The loss of sodium induces volume depletion and the retention of administered sodium chloride, similar to the events after bicarbonate loss, thereby causing a normal anion gap metabolic acidosis. As GFR falls in the course of renal failure, a portion of these inorganic anions is retained, leading to conversion to a mixed normal anion gap and high anion gap acidosis or dominant high anion gap acidosis. Thus, the major determinant of the electrolyte pattern in renal acidosis is the interplay between tubular dysfunction and GFR. Factors that preferentially impair tubular proton secretion without altering glomerular filtration, such as low aldosterone levels or tubular damage, will favor the development of normal anion gap acidosis (46).

If the accumulating acid contains an anion other than chloride, such as lactate in lactic acidosis or β-hydroxybutyrate in ketoacidosis, then the decrement in serum bicarbonate will be accompanied by an elevation in the unmeasured anion concentration (2,3,15,44). This type of metabolic acidosis, therefore, is termed high anion gap acidosis. High anion gap acidosis generally is due to overproduction of organic acids or the concomitant and proportionate reduction in the excretion of anions and net acid noted with various types of renal failure (2,46). In many cases, the identity of anions that contribute to the elevated anion gap can be determined (15,44). This is particularly true when the serum anion gap is >30 mEq/L (Δ mean AG >14 mEq/L (44), in which case the most common anions found are lactate (lactic acidosis) and β-hydroxybutyrate and acetoacetate (ketoacidosis). However, a lesser increase in the serum anion gap (anion gap 24 mEq/L or less) can be present without an identifiable, accumulating acid in >30% of cases (44). Others also have reported high anion gap forms of metabolic acidosis in which only a portion of the offending acids could be identified (47,48). Gabow et al. (44) studied the quantitative composition of the increased anion gap in 22 of the patients with high anion gap metabolic acidosis in detail: Approximately 67% of the increased anion gap in these patients could be explained by identifiable organic acids, approximately 13% was attributed to changes in serum albumin and phosphate concentrations, and, on average, approximately 20% remained unaccounted for. This disparity between the increase in the serum anion gap and the level of organic acid anions detected in the blood is perplexing and emphasizes the complexity of the relationship between the anion gap and changes in organic anion concentration.

A rise in the serum anion gap outside the upper limit of normal might not be realized in all patients with documented organic acidosis, indicating that the serum anion gap is an insensitive screen for mild to moderate organic acidosis (49). In one study, 100% of patients with a serum lactate concentration >10 mEq/L had a serum anion gap that exceeded 16 mEq/L, the upper limit of normal in their laboratory. However, only 50% of patients in whom serum lactate levels varied between 5 and 9.9 mEq/L, lactate levels that are associated with a mor-
tality that approached 75%, had an serum anion gap that exceeded 16 mEq/L (49).

Striking elevations in the serum anion gap, defined by some as values >45 mEq/L (Δ AG 30 mEq/L), are uncommon (20,43,48). Lokleha and Lokleha (20) found only 10 serum anion gap values that exceeded 45 mEq/L out of >7000 sets of electrolytes from all patients who were hospitalized during a single year (20). Moreover, in the prospective analysis of 57 patients with an elevated serum anion gap that was carried out by Gabow et al. (44), only one patient had a serum anion gap that exceeded this value. Although in this study the serum anion gap values that were >30 mEq/L largely were associated with identifiable forms of organic acidosis, it is surprising that in several cases in which the serum anion gap was markedly elevated (> 45 mEq/L) its rise could not be accounted for solely by a rise in organic acid anion concentration (48). Indeed, Oster et al. (48) reported a single patient with a serum anion gap of 57 mEq/L and reviewed nine other studies in which the serum anion gap ranged from 45 to 78 mEq/L. In all cases, the increases in measured organic anions were insufficient to explain the increased anion gap. Moreover, in several cases, increases in serum phosphate and albumin concentrations accounted for approximately 70 to 80% of the rise in the anion gap, with minimal or no change in organic anion concentration (43,48). As a whole, the extreme elevation in serum anion gap required the presence of renal failure, hemoconcentration, and increases in serum phosphorus and albumin concentrations, because accumulation of organic acid anions per se was insufficient to cause such a large increment in the serum anion gap. Therefore, a marked rise in the serum anion gap, although highly suggestive, does not automatically indicate the presence of organic acidosis.

The evolution of the increase in the serum anion gap and its relationship to changes in serum osmolality has been studied in intoxications, such as ethylene glycol intoxication and methanol intoxication (48,50,51). In these disorders, toxicity emanates from the metabolism of the ingested alcohols via the enzyme alcohol dehydrogenase into glycolic acid and formic acid, respectively (48,50,51). This process, although beginning relatively soon after ingestion, can be slow, particularly with methanol: t1/2 methanol to formic acid approximately 6 to 18 h (50). Therefore, acidosis can develop as late as 15 to 20 h after ingestion (50), a time when the concentration of these alcohols become undetectable. Therefore, the rise in the serum anion gap in these disorders occurs over time rather than developing rapidly, as often observed with lactic acidosis or ketoacidosis. Of interest, there can be an inverse relationship between changes in the serum osmolal gap and the serum anion gap, the serum osmolal gap (reflecting accumulation of osmotically active alcohols) falling as the serum anion gap (reflecting accumulation of organic acid anions) rises. This could explain the occasional failure to detect an elevated serum osmolal gap in patients with documented methanol or ethylene glycol intoxication (50,51).

This division of metabolic acidosis into disorders that are associated with a normal or a high anion gap classically has been a useful tool in differential diagnosis. Although useful diagnostically, the classification might be a marked oversimplification. As indicated in Table 3, disorders in which there is overproduction of acid (which therefore should manifest a high anion gap acidosis) can have a mixed high and normal anion gap acidosis or pure normal anion gap acidosis (46,52–54). Insights into this conundrum can be obtained by examining certain aspects of the evolution of the electrolyte pattern with different types of overproduction acidosis.

Within the first minutes to 1 h or so after the development of organic acidosis, the accumulating protons titrate serum bicarbonate. To maintain electroneutrality, the accompanying anion is retained. As a consequence, during this period, the rise in the unmeasured anion concentration and fall in serum bicarbonate concentration are equivalent, and there is a pure high anion gap metabolic acidosis (55,56). However, if the acidosis persists for more than a few hours, then reductions in serum bicarbonate concentration in excess of the increase in serum anion gap can occur (52,54) (i.e., some component of normal anion gap acidosis can develop). It has been postulated that this electrolyte pattern might emerge if urinary excretion of organic acid anions (potential base) outstrips the ability of the kidney to regenerate bicarbonate, the excreted anions being replaced by chloride (54,57,58). Indeed, the appearance of normal anion gap acidosis in overproduction acidosis has been shown to depend in large part on the fraction of the filtered anion that is excreted.

### Table 3. Serum anion gap in metabolic acidoses associated with overproduction or decreased excretion of protons and anions

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Serum Anion Gap*</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Ketoacidosis</td>
<td>Increased or normal</td>
<td>(52)</td>
</tr>
<tr>
<td>Lactic acidosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l-lactic acidosis</td>
<td>Most commonly increased; occasionally normal</td>
<td>(54)</td>
</tr>
<tr>
<td>d-lactic acidosis</td>
<td>Increased or normal</td>
<td>(61)</td>
</tr>
<tr>
<td>Toluene poisoning</td>
<td>Occasionally increased; most commonly normal</td>
<td>(58)</td>
</tr>
<tr>
<td>Methanol and ethylene glycol intoxication</td>
<td>Usually increased; can be normal</td>
<td>(50)</td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>Increased or normal</td>
<td>(46)</td>
</tr>
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</table>

*The serum anion gap will depend not only on nature of the acidosis but also on its duration and the quantity and the type of fluids given during its treatment.
A prototypical example is toluene poisoning. In this disorder, excess hippuric acid is produced initially, leading to buffering by plasma bicarbonate and a high anion gap acidosis. Subsequently, the serum anion gap can normalize and a dominant normal anion gap acidosis might emerge (58,59). The absence of an elevated anion gap in many patients, despite marked overproduction of hippuric acid, initially was attributed to development of renal tubular acidosis. However, subsequent investigations have indicated that it is due largely to urinary excretion of hippurate (approximately 100% of the filtered load) with accompanying sodium engendered by the volume depletion. A high anion gap acidosis can reemerge if the volume contraction leads to a fall in sodium engendered by the volume depletion. A high anion gap acidosis is said to coexist. By contrast, when the ΔAG exceeds the ΔHCO₃⁻, a metabolic alkalosis (or other hyperbicarbonatemic disorder) is said to coexist. The ΔAG also has been used to assess changes in organic acid concentration: An increase has been taken to indicate a rise in organic acid concentration presumably reflecting a rise in its production. However, as indicated by Table 3, a closer examination of the events that unfold during the evolution of high anion gap metabolic acidosis indicates that this 1:1 stoichiometry might be transient in nature and/or depend largely on the type of metabolic acidosis present (23,45,52,54,56,61,69).

Although the ΔAG/ΔHCO₃⁻ might be 1:1 (55,56) initially, the ratio potentially can change as the acidosis persists. First, a portion of the protons are buffered within compartments outside the extracellular fluid (23,70,71). Therefore, the generated anions also must disappear from the extracellular fluid at a similar rate to preserve the 1:1 stoichiometry (66). In addition, in response to metabolic acidosis, renal generation of bicarbonate increases, a process that begins almost immediately, but takes hours to days to reach its peak. Concomitantly, urinary excretion of the generated anions will occur, the magnitude of which depends on the interplay between their increased quantity in the filtrate and their tubular reabsorption. Thus, to preserve a 1:1 stoichiometry that is present in the early stages of metabolic acidosis, the rates of proton and anion exit from the extracellular fluid, as well as those of the renal generation of new base and excretion of filtered anions must be roughly equal throughout the subsequent course of the metabolic acidosis. Alternatively, if the rates of proton exit from the extracellular fluid is greater than that of anions, causing the ratio of ΔAG to ΔHCO₃⁻ bicarbonate to differ from 1:1, then this ratio still can approach 1:1 if the urinary excretion of anions outstrips the generation of bicarbonate (52,54,64,70).

Perusal of published clinical and experimental studies of the most common disorders that are associated with accumulation of organic acids, ketoacidosis and lactic acidosis, as well as more rare causes of organic acid accumulation, such as toluene poisoning, reveals in fact that there is variable stoichiometry of the ΔAG/ΔHCO₃⁻ (48,52–54,58,70,72,73). It should be emphasized that virtually all clinical studies have used mean normal values for serum anion gap and plasma bicarbonate concentration, rather than the actual normal values of individual patients, for calculation of the ΔAG/ΔHCO₃⁻. It is likely that this practice has an important impact on the computation of the ratio and the derived pathophysiologic conclusions.

α-Lactic Acidosis (Type A). The causes of lactic acidosis are diverse, with hypoxia and reduced tissue perfusion being the main pathogenic factors underlying the most common form
of lactic acidosis (57). In some clinical cases and experimental studies of this type of lactic acidosis, $\Delta AG/\Delta HCO_3^-$ was 1:1 (55–57), whereas in others it was greater than 1:1 (54,71) in the absence of apparent coexisting metabolic alkalosis or other hyperbicarbonatemic disorder (range 1.6 to 1.8) (54,57,63,71). This deviation from the 1:1 stoichiometry has been postulated by some to result from a disparity between the rates of entry of protons and lactate anions into cellular compartments (32,70,71), whereby protons are buffered outside the extracellular fluid but the lactate tends to remain within the extracellular fluid compartment. Others have suggested that expansion of the extracellular compartment (secondary to extrusion of cellular cation noted during the buffering process of accumulating acid) with resultant dilution of serum chloride might contribute to this disparity (71). The duration of the lactic acidosis, a determinant of cellular buffering, seems to affect the stoichiometry. Within the first 60 min of onset, the mean $\Delta AG/\Delta HCO_3^-$ in both animal and human studies is close to 1:1 (55,56,71). As the acidosis persists beyond a few hours, the mean ratio can rise to 1.8 (63,71). Because lactate excretion by the kidney with hypoxic lactic acidosis is low (fractional excretion approximately 4.5%), this ratio of $\Delta AG/\Delta HCO_3^-$ can remain elevated beyond 1 during the course of the acidosis (54).

Brivet et al. (53) found variability in the values for the $\Delta AG/\Delta HCO_3^-$ of their patients with l-lactic acidosis. These investigators examined the serum anion gap immediately after cessation of seizures in 35 patients. This ratio varied from 2:1 to <0.8:1. Approximately 33% of patients had a ratio <0.8, indicating the presence of normal anion gap acidosis. No other cause of normal anion gap acidosis could be documented. Similarly, Hatherill et al. (74) found that 63% of 46 children who were in shock and were admitted to the intensive care unit had normal anion gap acidosis, and Iberti et al. (49) reported that >50% of patients with a significant elevation in serum lactate concentration and hypobicarbonatemia had a serum anion gap within the range of normal. Taken as a whole, these studies demonstrate that the $\Delta AG/\Delta HCO_3^-$ in hypoxic lactic acidosis can be variable. A ratio greater than 1 is common and should not cause the physician to assume automatically that metabolic alkalosis coexists. Although less frequent, a ratio of <0.8 might be present in the absence of other disorders that produce normal anion gap acidosis.

D-Lactic Acidosis. The $\Delta AG/\Delta HCO_3^-$ in d-lactic acidosis in many studies has been found to be more variable than in l-lactic acidosis (60,61). Consistent with this variability, various types of metabolic acidosis can be found, including high anion gap, mixed high anion gap, and normal anion gap acidosis, or normal anion gap acidosis alone (60,61).

The common occurrence of normal anion gap acidosis in d-lactic acidosis has been attributed to efficient excretion of the d-lactate anion with sodium during the course of the disorder. As described previously, d-lactate excretion is relatively high because the renal tubular lactate transporter binds d-lactate less well than l-lactate, the reabsorption of the d-isomer being substantially lower (renal threshold <1 mmol/L (60,61).

Ketosis. In diabetic ketoacidosis, the $\Delta AG/\Delta HCO_3^-$ that is determined on admission to the hospital and during the course of treatment has been reported to vary from approximately 1:1 to <0.8 (52,54,62,72). As a consequence, despite overproduction of non–Cl-containing acids, a mixed normal anion gap and high anion gap acidosis or pure normal anion gap acidosis can be present (52,54,70). Although it has been speculated that a difference in the apparent volume of distribution of protons and ketone anions could account for development of a normal anion gap acidosis (63), studies by Adrogue et al. (52,72) indicate that this occurrence more likely is due to renal excretion of generated anions in excess of new bicarbonate synthesis (52,72). Because this process is kidney dependent, it requires relatively preserved GFR. Therefore, patients with volume depletion and renal insufficiency were more likely to have a high anion gap metabolic acidosis, whereas those with relatively preserved extracellular volume and essentially intact renal function developed normal anion gap acidosis (52).

Further support for the importance of urinary anion excretion in the development of normal anion gap metabolic acidosis can be gleaned from studies of Kim et al. (54). These authors documented that fractional excretion of ketones was high, approximately 45%, 10-fold greater than that of l-lactate (approximately 4.5%) in lactic acidosis, which commonly is associated with a high anion gap metabolic acidosis, but lower than that of toluene (approximately 100%) in toluene intoxication (noted with glue sniffing), which often is associated with normal anion gap acidosis (see the Toluene Intoxication section).

Similar findings were reported by Elsaf et al. (75), who examined the prevalence of various acid-base disorders in 40 patients with ketoacidosis. Although many patients had only high anion gap metabolic acidosis, several had either high anion gap and normal anion gap acidosis or the concurrence of high anion gap metabolic acidosis and metabolic alkalosis. In many of the latter cases, disorders that usually are associated with the development of normal anion gap acidosis or metabolic alkalosis were not present. Studies of patients with alcoholic ketoacidosis also have demonstrated variability in the $\Delta AG/\Delta HCO_3^-$ (76). Thus, of 40 patients with documented alcoholic ketoacidosis, 19 (49%) had a $\Delta AG/\Delta HCO_3^-$ close to 1:1, consistent with a high anion gap metabolic acidosis, whereas 11 (26%) patients had a ratio that exceeded 1:1, which was interpreted as reflecting a combined metabolic acidosis and alkalosis. In 10 (25%) patients, the $\Delta HCO_3^-$ exceeded the $\Delta AG$, suggesting the coexistence of a high anion gap and normal anion gap acidosis.

Toluene Intoxication. The $\Delta AG/\Delta HCO_3^-$ with toluene intoxication most frequently is <0.8. Less often, this ratio can approach 1:1, particularly early in its course. Thus, the metabolic acidosis of toluene intoxication usually is of the normal anion gap variety, although mixed or dominant high anion gap acidosis also can be seen (77,78). The normal anion gap acidosis, in the face of marked overproduction of hippuric acid, can be ascribed to efficient urinary excretion of hippurate (approximately 100% of the filtered load) with accompanying sodium and potassium (54,58).

Metabolic Alkalosis. Metabolic alkalosis, particularly that due to vomiting or diuretic use, can be associated with a small
increment in the serum anion gap, approximately 4 to 6 mEq/L, in the absence of disorders that might increase it, such as organic acidosis or renal failure (42,45). The increment in the serum anion gap has been attributed largely to the increased serum albumin concentration, with a smaller component being due to the increase in anionic equivalency of proteins consequent to the rise in blood pH (42,45). The dominance of changes in protein concentration rather than the titration of plasma proteins in inducing alterations in the serum anion gap was supported by studies of Paulson et al. (79). In experimental studies, they examined the effects of acute changes in blood pH induced by hypercapnia and hypocapnia on serum anion gap in dogs and also reviewed the relevant literature. They found that for every 0.1-unit change in blood pH, the serum anion gap rose by only 0.15 mEq/L. These observations suggest that acute titration of circulating proteins in itself is insufficient to induce large changes in the serum anion gap with metabolic alkalosis.

Respiratory Alkalosis. Serum anion gap does not change notably in acute respiratory alkalosis (79), but small increases (on the order of 3 mEq/L for a 20-mmHg chronic reduction in PaCO₂) have been observed in chronic respiratory alkalosis (80). Titration of plasma proteins can account only for a trivial component of such an increase, and no appreciable change in plasma lactate occurs during chronic hypocapnia. Therefore, the source of the small increase in the serum anion gap remains unidentified.

IgA Paraproteinemia. As noted previously, the accumulation of paraproteins in the blood can cause an alteration in the serum anion gap (3–5). Therefore, because IgA paraproteins have isoelectric points below the physiologic pH, they will behave as anions in the blood (26). The impact of IgA myeloma on serum anion gap has been conflicting. DeTroyer et al. (26) found no increment in the serum anion gap in patients with IgA myeloma and no correlation between the concentration of this paraprotein and the serum anion gap. By contrast, Paladini et al. (5) found a slight increment in the mean value of the serum anion gap. However, several patients had values within the normal range. There was a direct correlation between the concentration of paraprotein and the serum anion gap. From these studies, one can infer that although an elevated serum anion gap can be seen with IgA myeloma, this finding is not as consistent as the obverse change that is noted with IgG myeloma.

Laboratory Error. As with a low serum anion gap, an elevated serum anion gap can result, of course, from laboratory error. However, the frequency of an elevated anion gap as a result of laboratory error has not been reported. This might reflect less emphasis on this cause of an anion gap compared with a low value. Overestimations of sodium and underestimations of chloride and bicarbonate theoretically can result in an elevated anion gap. If the serum is separated and left exposed to the air for more than 1 h, then bicarbonate concentration will be spuriously low, thereby elevating the anion gap (81).

Severe Hyperphosphatemia. Several case reports of patients with severe hyperphosphatemia associated with exogenous phosphate administration have been reported (43,82). In these cases, serum phosphate concentration was approximately 19 to 23 mg/dl and the serum anion gap was ≥50 mEq/L. Calculation by the authors revealed that the increased serum phosphate concentration could account for approximately 60% of the increased serum anion gap, whereas inclusion of changes in albumin concentration increased this percentage to approximately 80%. Review of the literature by Kirschbaum (43) revealed that severe hyperphosphatemia, per se, is an important factor in marked elevations in the serum anion gap. Therefore, in the absence of renal failure or retention of organic acid anions, severe hyperphosphatemia should be considered in the differential diagnosis of an elevated serum anion gap.

Change in Valence of Circulating Proteins. Kamel et al. (66) described a volume-contracted patient who had high anion gap acidosis and in whom neither identifiable accumulating anions nor increases in the serum albumin and phosphate concentrations could be detected. Experimental studies that simulated the clinical condition of the patient in rats confirmed a rise in the serum anion gap, the composition of which could not be explained. These investigators postulated that under these circumstances, the valence of anionic proteins was altered to preserve extracellular volume via a Donnan effect. The frequency of this mechanism as an explanation for an increased serum anion gap is unknown.

Conclusion
The serum anion gap is a calculated entity that has been used for more than 40 years to assess acid-base disorders; assess the quality of laboratory determinations; and detect disorders such as paraproteinemias and intoxications with bromide, lithium, or iodide. Furthermore, the relationship between the change in the anion gap and serum bicarbonate concentration (ΔAG/ΔHCO₃⁻) is noted with metabolic acidosis has been used to detect the presence of complex metabolic acid-base disorders, such as the combination of high and normal metabolic acidosis or metabolic alkalosis. Although the serum anion gap remains a valuable clinical tool, appreciation of its limitations should make the clinician cautious in its interpretation. The wide interindividual variability makes it difficult to interpret correctly the importance of small deviations from normal in the absence of knowledge of the baseline serum anion gap for the patient who is being evaluated. The classification of metabolic acidosis into two categories, high anion gap and normal anion gap, generally is useful in the rapid diagnosis of the cause of the metabolic acidosis. However, recent studies indicate significant overlap between the categories: Specifically, patients with presumed high anion gap metabolic acidosis might manifest a significant degree of normal anion gap acidosis without evidence of other disorders that can produce a normal anion gap acidosis. In this regard, the variability in the ΔAG/ΔHCO₃⁻ in various types of high anion gap metabolic acidosis hampers the ability of the clinician to describe precisely the nature of the acid-base disorders that are present by using this information in isolation. For example, a patient who has a high anion gap metabolic acidosis and in whom the ΔAG/ΔHCO₃⁻ is 1.6 or greater might be suspected of having combined metabolic aci-
dosis and alkalosis (or other hyperbicarbonatemic disorder), when this ratio merely reflects a disparity in the volume of distribution of protons and the accompanying anion. Similarly, if the $\Delta \text{HCO}_3^-$ is substantially greater than the $\Delta \text{AG}$, then it does not necessarily reflect the presence of disorders that produce a normal anion gap acidosis (or other hypobicarbonatemic disorder), because some component of normal anion gap acidosis is present in several organic acidoses.

As with all disorders, therefore, scrutiny of other information, such as the history, physical examination, and other laboratory data, should be considered in making a diagnosis of an acid-base disorder. With these caveats in mind, it is important to emphasize that the serum anion gap remains an inexpensive and effective method of detecting or suspecting the presence of various disorders. Therefore, it should be continued to be used in the evaluation of patients.

**Disclosures**

None.

**References**

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